

On page 14, Table 1, fourth primer, insert -- (SEQ ID NO:6)-- before "+69"

On page 14, Table 1, fifth primer, insert -- (SEQ ID NO:7)-- before "-90"

On page 14, Table 1, sixth primer, insert -- (SEQ ID NO:8)-- before "+1203"

On page 14, Table 1, seventh primer, insert -- (SEQ ID NO:9)-- before "+893"

On page 14, Table 1, eighth primer, insert -- (SEQ ID NO:10)-- before "+1094"

On page 14, Table 1, ninth primer, insert -- (SEQ ID NO:11)-- before "-83"

In FIG. 1, last line, insert -- (SEQ ID NO:12)-- before "1269"

In FIG. 1, insert -- (SEQ ID NO:13)-- after the "\*" on the line ending with "365"

No fees are believed due at this time, however, please charge any deficiencies or credit any overpayments to deposit account number 10-0435 with reference to our attorney docket number (21416/91513)

Respectfully submitted,



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**Example 1: An Assay For Resistant Swine**

The polymorphisms of the present invention are easily identified using PCR-RFLP tests. One embodiment of the tests used a 160bp fragment of porcine alpha (1,2) fucosyltransferase 1 amplified using PCR with the following primers;

5 5'CCAACGCCTCCGATTCTGT3' --(SEQ ID NO: 1)-- and 5'GTGCATGGCAGGCTGGATGA3' --(SEQ ID NO: 2)--. Preferred PCR conditions for this embodiment are 25 cycles at the following times and temperatures: 94 °C, 30 sec; 60 °C, 45 sec; 72 °C, 90 sec. The amplified DNA from resistant swine was digested by the restriction enzyme Hgal, but was not digested by the restriction enzyme HinPI. The 10 amplified DNA from homozygous susceptible swine was digested by the restriction enzyme HinPI. The amplified DNA from heterozygous susceptible swine was partially digested by both enzymes.

Alternatively, DNA was isolated from porcine nucleated cells according to standard procedures. Direct sequencing of porcine *FUT1* and *FUT2* sequences and their flanking regions in animals of different *ECF18R* genotype (Bb, bb) resulted in the identification of two G --> A transitions at positions 307 and 857 (termed *M307* and *M857*, respectively) of the *FUT1* ORF. The *M307* transition eliminates a restriction site for CfoI. Amplification of DNA isolated from porcine nucleated cells was performed according to standard procedures with primers P6 and P11 (3 min at 95 °C, 30 cycles of 20 30 sec at 95 °C, 30 sec at 56 °C and 30 sec at 72 °C, followed by a 7 min final extension at 72 °C) followed by CfoI digestion and separation on a 3% agarose gel resulted in a restriction fragment length polymorphism (RFLP). Homozygous *M307<sup>AA</sup>* animals showed 2 bands. Homozygous *M307<sup>GG</sup>* animals showed 93-, 241- and 87bp fragments. Heterozygous animals showed all four fragments.

25 **Example 2: Sensitivity and Specificity Of An Assay Using Alpha (1,2) Fucosyltransferase In Detecting Swine Resistant to F18 E. coli**

A study was conducted to determine the association between disease resistance and the polymorphism at position 307 of the *FUT1* gene. 183 weaned swine (ranging in 30 ages 2-6 months) were obtained from six different breeding herds. Only one of these herds was known to contain resistant animals before the start of the study, and this herd is known to have a high incidence of porcine stress syndrome. The other 5 herds had no evidence of porcine stress syndrome, and the incidence of disease resistance was unknown. Swine from each herd were randomly selected, humanely euthanized and



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O I P E J C 69  
C A N C E L L E D

Table 1: Sequences Of Forward-(F) And Reverse-(R) Primers And Their Relative Position to the Porcine *FUT1* and *FUT2* Start Codons<sup>2</sup>

	Primer name	Primer Sequence	Position
5	FUT1 P6 (R)	5'-CTTCAGCCAGGGCTCCTTAAG-3' --(SEQ. ID NO:3)--	+489
	FUT1 P7 (F)	5'-TTACCTCCAGCAGGCTATGGAC-3' --(SEQ ID NO: 4)--	+720
	FUT1 P10 (R)	5'-TCCAGAGTGGAGACAAGTCTGC-3' --(SEQ ID NO: 5)--	+1082
	FUT1 P11 (F)	5'-CTGCCTGAACGTCTATCAAGATC-3' --(SEQ ID NO: 6)--	+69
	FUT1 P16 (F)	5'-AGAGTTTCCTCATGCCACAGG-3' --(SEQ ID NO: 7)--	-90
10	FUT1 P18 (R)	5'-CTGCTACAGGACCACAGCATC-3' --(SEQ ID NO: 8)--	+1203
	FUT1 PBEST (R)	5'-ACCAAGCAGCGCAAAGTCCCTGAC GGGCACGGCCTC-3' --(SEQ ID NO: 9)--	+893
	FUT2 P16 (R)	5'-CTCCCTGTGCCTTGGAAAGTGAT-3' --(SEQ ID NO: 10)--	+1094
	FUT2 P17 (F)	5'-AACTGCACTGCCAGCTTCATGC-3' --(SEQ ID NO: 11)--	-83

15 Table 2: Overall Recombination Fractions ( $\theta$ ), Lodscores (Z) And Number Of Informative Animals (N) For *M307* And Loci Of The *HAL* Linkage Group In The Landrace Experimental Population

	Locus pair	N	$\theta$	Z
20	<i>S-ECF18R</i>	183	0.01	50.6
	<i>M307-S</i>	183	0.01	50.6
	<i>M307-ECF18R</i>	216	0.01	57.1
	<i>M307-RYR1</i>	198	0.02	47.2
	<i>M307-GP1</i>	147	0.03	34.2
25	<i>M307-PGD</i>	147	0.04	24.5 -

<sup>2</sup> Primers *FUT1* P10 and *FUT1* P11 are derived from the human *FUT1* gene.

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FIGURE 1

M	W	V	P	S	R	R	H	L	C	L	T	F	L	L	V	C	17				
CT	CGA	GCC	ATG	TGG	GTC	CCC	AGC	CGC	CGC	CAC	CTC	TGT	CTG	ACC	TTC	CTG	CTA	GTC	TGT	59	
<u>V</u>	L	A	A	I	F	F	L	N	V	Y	Q	D	L	F	Y	S	G	L	D	37	
GTT	TTA	GCA	GCA	ATT	TTC	TTC	CTG	AAC	GTC	TAT	CAA	GAC	CTC	TTT	TAC	AGT	GGC	TTA	GAC	119	
L	L	A	L	C	P	D	H	N	V	V	S	S	P	V	A	I	F	C	L	57	
CTG	CTG	GCC	CTG	TGT	CCA	GAC	CAT	AAC	GTG	GTA	TCA	TCT	CCC	GTG	GCC	ATA	TTC	TGC	CTG	179	
A	G	T	P	V	H	P	<u>N</u>	A	S	D	S	C	P	K	H	P	A	S	F	77	
GCG	GGC	ACG	CCG	GTA	CAC	CCC	AAC	GCC	TCC	GAT	TCC	TGT	CCC	AAG	CAT	CCT	GCC	TCC	TTT	239	
S	G	T	W	T	I	Y	P	D	G	R	F	G	N	Q	M	G	Q	Y	A	97	
TCC	GGG	ACC	TGG	ACT	ATT	TAC	CCG	GAT	GGC	CGG	TTT	GGG	AAC	CAG	ATG	GGA	CAG	TAT	GCC	299	
T	L	L	A	L	A	Q	L	N	G	R	Q	A	F	I	Q	P	A	M	H	117	
ACG	CTG	CTG	GCC	CTG	<del>GCG</del>	CAG	CTC	AAC	GGC	CGC	CAG	GCC	TTC	ATC	CAG	CCT	GCC	ATG	CAC	359	
A	V	L	A	P	V	F	R	I	T	L	P	V	L	A	P	E	V	D	R	137	
GCC	GTC	CTG	GCC	CCC	GTG	TTC	CGC	ATC	ACG	CTG	CCT	GTC	CTG	GCG	CCC	GAG	GTA	GAC	AGG	419	
H	A	P	W	R	E	L	E	L	H	D	W	M	S	E	D	Y	A	H	L	157	
CAC	GCT	CCT	TGG	CGG	GAG	CTG	GAG	CTT	CAC	GAC	TGG	ATG	TCC	GAG	GAT	TAT	GCC	CAC	TTA	479	
K	E	P	W	L	K	L	T	G	F	P	C	S	W	T	F	F	H	H	L	177	
AAG	GAG	CCC	TGG	CTG	AAG	CTC	ACC	GGC	TTC	CCC	TGC	TCC	TGG	ACC	TTC	TTC	CAC	CAC	CTC	539	
R	E	Q	I	R	S	E	F	T	L	H	D	H	L	R	Q	E	A	Q	G	197	
CGG	GAG	CAG	ATC	CGC	AGC	GAG	TTC	ACC	CTG	CAC	GAC	CAC	CTT	CGG	CAA	GAG	GCC	CAG	GGG	599	
V	L	S	Q	F	R	L	P	R	T	G	D	R	P	S	T	F	V	G	V	217	
GTA	CTG	AGT	CAG	TTC	CGT	CTA	CCC	CGC	ACA	GGG	GAC	CGC	CCC	AGG	ACC	TTC	GTG	GGG	GTC	659	
H	V	R	R	G	D	Y	L	R	V	M	P	K	R	W	K	G	V	V	G	237	
CAC	GTG	CGC	CGC	GGG	GAC	TAT	CTG	CGT	GTG	ATG	CCC	AAG	CGC	TGG	AAG	GGG	GTG	GTG	GGT	719	
D	G	A	Y	L	Q	Q	A	M	D	W	F	R	A	R	Y	E	A	P	V	257	
GAC	GGC	CGT	TAC	CTC	CAG	CAG	GCT	ATG	GAC	TGG	TTC	CGG	GCC	CGA	TAC	GAA	GCC	CCC	GTC	779	
F	V	V	T	-	S	N	G	M	E	W	C	R	K	N	I	D	T	S	R	G	277
TTT	GTG	GTC	ACC	AGC	AAC	GGC	ATG	GAG	TGG	TGC	CGG	AAG	AAC	ATC	GAC	ACC	TCC	CGG	GGG	839	
D	V	I	F	A	G	D	G	R	E	A	A	P	A	R	D	F	A	L	L	297	
GAC	GTG	ATC	TTT	GCT	GGC	GAT	GGG	<del>CGG</del>	GAG	GCC	GGC	CCC	GCC	AGG	GAC	TTT	GCG	CTG	CTG	899	
V	Q	C	<u>N</u>	H	T	I	M	T	I	G	T	F	G	F	W	A	A	Y	L	317	
GTG	CAG	TGC	AAC	CAC	ACC	ATC	ATG	ACC	ATT	GGC	ACC	TTC	GGC	TTC	TGG	GCC	GCC	TAC	CTG	959	
A	G	G	D	T	I	Y	L	A	<u>N</u>	F	T	L	P	T	S	S	F	L	K	337	
GCT	GGT	GGA	GAT	ACC	ATC	TAC	TTG	GCT	AAC	TTC	ACC	CTG	CCC	ACT	TCC	AGC	TTC	CTG	AAG	1019	
I	F	K	P	E	A	A	F	L	P	E	W	V	G	I	N	A	D	L	S	357	
ATC	TTT	AAA	CCC	GAG	GCT	GGC	TTC	CTG	CCC	GAG	TGG	GTG	GGC	ATT	AAT	GCA	GAC	TTG	TCT	1079	
P	L	Q	M	L	A	G	P	--	(SEQ ID NO:13)	--										365	
CCA	CTC	CAG	ATG	TTG	GCT	GGG	CCT	TGA	ACC	AGC	CAG	GAG	CCT	TTC	TGG	AAT	AGC	CTC	GGT	1139	
CAA	CCC	AGG	GCC	AGC	GTT	ATG	GGT	CTC	CGG	AAG	CCC	GAG	TAA	CTT	CCG	GAG	ATG	CTG	GTG	1199	
GTC	CTG	TAG	CAG	GCT	GGA	CAC	TTA	TTT	CAA	GAG	TGA	TTC	TAA	TTG	GCT	GGA	CTC	AGA	GGA	1259	
AAC	CCT	GCA	G	--	(SEQ ID NO:12)	--														1269	